Supplemental Methods

Subjects

Male Sprague-Dawley rats, weighing 250-275g at the start of the experiment, were individually housed in a temperature and humidity controlled vivarium on a reversed light-dark cycle. Food and water were freely available, except when specified. All experiments were conducted during the dark portion of the circadian cycle, and in accordance with the specifications of the National Institute of Health *Guide for the Care and Use of Laboratory Animals*. Rats were weighed and handled daily for one week prior to surgery.

Food Training

Rats were trained to lever press on a fixed ratio (FR)1 schedule of food reinforcement (45 mg pellets; Noyes, Lancaster, NH) in operant conditioning chambers (30 x 20 x 24 cm high; Med Associates Inc., St. Albans, VT) during a 16-h overnight food training session. The chambers were equipped with two retractable levers, a stimulus light above each lever, a food pellet dispenser between the levels, and a house light on the wall opposite to the levels. During the session, each press on the active lever resulted in delivery of a food pellet only. Lever presses on the inactive level had no programmed consequence.

Surgery

Rats were anesthetized with ketamine HCl (87.5 mg/kg Ketaset, Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (5 mg/kg Rompum, Bayer, Shawnee Mission, KS) and implanted with indwelling jugular catheters as described previously(McFarland and Kalivas, 2001). For catheter implantation, a guide cannulae (C313G, Plastics One) attached to silastic tubing (.025 ID, .047 OD, VWR) and Marlex mesh via dental cement were inserted subcutaneously between the shoulder blades, with the guide cannula externalized through a dermal biopsy hole (3mm). The other end of the silastic tubing was threaded subcutaneously, inserted into the jugular vein, and secured in place with sutures to the underlying muscle tissue. The catheter was flushed daily with heparinized saline (0.2ml of 100 IU) and cefazolin antibiotic (0.2 ml of 0.1g/ml). Following surgery, subjects were allowed 7 days to heal before beginning behavioral training. On the sixth day, subjects were placed on a food ration of 20-25 g per day (Purina Rat Chow) for the remainder of the experiment. Additionally, they were tested for catheter patency with an intravenous infusion of Brevital (0.15 ml of 1% methohexital sodium; Eli Lilly Co., Indianapolis, IN). Rats that did not demonstrate an immediate loss of the righting reflex for approximately 2 min following the infusion were excluded for the experiment. A total of 47 animals undergoing surgery completed the study

Heroin self-administration

Rats were trained to self-administer heroin in operant chambers under an FR-1 schedule for 3-h daily session for 12 consecutive days. Pressing on the active level resulted in the infusion of heroin (0.1 mg per infusion for day 1-2, 0.05 mg per infusion

for day 3-4, 0.025 mg per infusion for day 5-12; NIDA, Rockville, MD) prepared in a vehicle of 0.9% sterile saline over 4 sec as described previously (Fuchs and See, 2002). Each training session began when rats were connected to the drug delivery apparatus, the house light was illuminated and the two levers were inserted into the chamber. The delivery of each heroin infusion was accompanied with the light cue located above the active lever, and followed by a 20 sec. time out period during which responses on the active lever were counted but resulted in no reinforcement.

Chronic pretreatment with N-acetylcysteine

After the last session of self-administration, the rats were divided into the two groups. One group of rats (n=24) was pretreated chronically with N-acetylcysteine (100 mg/kg, i.p.; Sigma, St.Louis, MO) at 2.5 hrs before the extinction training and reinstatement testing for 15 consecutive days, the optimal dose and duration of N-acetylcysteine was chosen according to the previous report (Baker et al., 2003). Another group of rats (n=23) was pretreated with the saline vehicle using a same volume (2.0 ml/kg, i.p.).

Extinction Training

After heroin self-administration for 12 days, the rats underwent the extinction training for daily 3-h without any lights and drugs for consecutive 10 days in the operant chamber. During extinction sessions, responding on the active lever was recorded but had no program consequence. Three animals in each group only underwent 7 days of extinction training followed by 3 days in the home cage before beginning the cue reinstatement trial. However, all 6 animals continued daily NAC or vehicle injections while in the home cage.

Cue-induced or heroin-induced reinstatement

After extinction training, the rats were placed in the operant chambers for 3-h to test the reinstatement of heroin seeking induced by heroin-associated cues, including a house light that previously predicted drug availability and a 20 sec light cue that was associated with heroin infusion (Zhou et al., 2007). The house light was illuminated and the levers inserted into the chamber. Every active lever presses resulted in the presentation of light cue. The rats were the returned to the home cage for 3 days. The next day, the rats were injected heroin (0.25 mg/kg, s.c.) in the operant chamber to induce reinstatement. Lever presses were counted but resulted in no programmed consequences.

After the two reinstatement tests, the subjects were placed in the home chamber for 40 days without N-acetylcysteine, vehicle or heroin. Reinstatement was then induced by the light cue, and after an additional 4 days in the home cage, reinstatement induced again by heroin as described above. However, during these last reinstatement trials, no pretreatment with NAC or saline was administered.

References

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